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D UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
A61K 39/00

A1

(11) International Publication Number: WO 94/01130

(43) International Publication Date: 20 January 1994 (20.01.94)

(21) International Application Number: PCT/GB93/01459

(22) International Filing Date: 13 July 1993 (13.07.93)

(30) Priority data:
9214871.7 13 July 1992 (13.07.92) GB

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(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

DOC

(54) Title: A MAJOR HISTOCOMPATIBILITY COMPLEX ANTIGEN FOR USE AS VACCINE AGAINST AN IMMUNODEFICIENCY VIRUS

(57) Abstract

A major histocompatibility complex class I antigen is useful as a vaccine against an immunodeficiency virus. The antigen may be a human class I antigen such as an HLA-A, HLA-B or HLA-C antigen or β_2 microglobulin. The virus may be a human immunodeficiency virus (HIV) such as HIV-1 or HIV-2.

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VACCINES

This invention relates to vaccines against immunodeficiency viruses.

Cynomolgus macaques vaccinated with either inactivated partially purified simian immunodeficiency virus (SIV), fixed SIV-infected C8166 (a human T lymphoblastoid cell line) cells or fixed uninfected C8166 cells can be protected against a challenge infection with the 32H isolate of SIVmac 251 (grown in C8166)[1,2]. Protection is correlated with the levels of antibody response to cellular antigens in the human cells from which the virus immunogen was grown [2-6]. However, the mechanism of protection is unclear.

We have analyzed sera from these monkeys. We have found that there is positive correlation of protection with antibody response to the HLA class I molecule. Furthermore, sera from protected monkeys inhibited xenoantigen but not alloantigen-induced monkey T cell proliferation in vitro and the inhibition could be blocked by adsorption with murine P815 cells transfected with human HLA class I genes.

Accordingly, the invention provides a major histocompatibility complex (MHC) class I antigen for use in a method of treatment of the human or animal body by therapy, in particular for use as a vaccine against an immunodeficiency virus.

The invention also provides a pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and, as active ingredient, a MHC class I antigen. The invention further provides use of a MHC class I antigen in the manufacture of a medicament for use as a vaccine against an immunodeficiency virus.

The antigen is preferably a human class I antigen. The class I molecule may therefore be HLA-A, HLA-B or HLA-C or β_2-microglobulin (β_2m) which is the β-chain of the HLA class I molecule. The antigen may be the entire

class I molecule in which the heavy chain is HLA-A, HLA-B or HLA-C. These are known antigens and can be obtained in purified form. They may be prepared as recombinant proteins.

Alternatively, the class I antigen may be given presented by transfected cells, i.e. by cells transfected with a gene encoding the antigen and which consequently express the antigen. Transfected cells which may be administered to a human may be transfected cells of a human diploid cell line. Such cell lines have been tested for safety for the purpose of human vaccine manufacture. An appropriate cell line is the MRC5 cell line.

Allogenic lymphocytes which present a class I antigen may be administered to a patient. The lymphocytes may be given as live cells, for example as a blood transfusion. Alternatively they may also be given as fixed or inactivated cells. The lymphocytes may be ones in which the expression of the class I antigen has been enhanced, for example by stimulation with a mitogen or gamma-interferon.

The antigen may be used to vaccinate a host against an immunodeficiency virus. The host may be a human or animal but typically it will be wished to vaccinate a human against a human immunodeficiency virus (HIV). That virus may be HIV-1 or HIV-2. A prophylactic treatment for disease states attributable to infection by an immunodeficiency virus can therefore be provided. The class I antigen may in particular act as an AIDS vaccine.

An effective amount of the antigen is administered to a host it is wished to vaccinate. The antigen can be given parenterally, for example subcutaneously or intramuscularly. The amount of antigen per dose depends on a variety of factors such as the age and the condition of the subject involved. A parenteral dose typically consists of from 20 μg to 1 mg of antigen, for example from 50 to 500 μg of antigen. A number of

doses may be given, for example from 2 to 4 doses over a period of up to six months. Each dose may be given one or two months apart.

An agent for use as a vaccine against an immunodeficiency virus is therefore provided. A pharmaceutical composition also comprising a pharmaceutically acceptable carrier or diluent can be formulated. The composition is thus sterile and pyrogen-free. The composition may also comprise an adjuvant such as $Al(OH)_3$ or saponin.

Compositions for intramuscular or subcutaneous injections may contain together with the antigen a pharmaceutically acceptable carrier, e.g. sterile water, olive oil, ethyl oleate, glycols e.g. propylene glycol, and if desired, a suitable amount of lidocaine hydrochloride.

The MHC class I antigens can be safely used by virtue of their negligible toxicity.

The following Examples illustrate the invention. In the accompanying drawings:

Figures 1a - d show the results of radioimmunoprecipitation followed by SDS-PAGE, and

Figure 1e shows the result of flow cytometry analysis.

Example 1

1. Monkeys were vaccinated as follows: I179-182, 4 x 500 μg formalin-fixed SIVmac 251 (32H isolate; 11/88 pool) + SAF-1 adjuvant subcutaneously (sc); J134-137, 3 x 500 μg formalin-fixed SIV mac 251 + RIBI adjuvant[36], sc; J138-141, 4 x 100 μg formalin-fixed SIVmac 251 + RIBI, sc; I217-220 and J68-71, 4 and 2 doses respectively of 2 x 10^6 SIVmac 25-1 infected C8166 cells fixed with glutaraldehyde + Quil-A adjuvant, sc; J72-75, 2 doses of 2 x 10^6 C8166 cells fixed with glutaraldehyde + Quil-A adjuvant, sc. All the animals were challenged intravenously with ten 50% monkey infectious doses (MID_{50}) SIVmac 32H isolate, 11/88 pool (grown in C8166 cells) 1

week after the final boost.

With the exception of monkeys J68-70, J72 and J73, the protected animals were not given a booster vaccination before rechallenge with the same preparation of virus stock. Protection was determined using the previously described method of polymerase chain reaction (PCR) for SIVmac proviral DNA with gag, pol as primers[7] as well as by virus isolation using direct cocultivation of monkey peripheral blood mononuclear cells (PBMC) with C8166 cells. Virus was detected by the appearance of cytopathic effects (CPE) and confirmed by immunfluorescence of virus antigen on infected cells using standard methods. Cultures which did not show CPE were maintained for at least 28 days before being discarded as negative.

a-d, Actively dividing C8166 cells (20-30 x 10^6) were labelled for 6 hr with 0.5mCi ^{35}S-methionine in methionine-free RPMI medium containing 10% foetal calf serum (FCS), lysed and radioimmune precipitated as previously described[8]. The washed immune complexes were reduced and electrophoresed in a 12.5% acrylamide gel. Gels were treated with Amplify (Amersham), dried and exposed to Kodak X-Omat RP film at -70°C.

e, One hundred μl of a suspension of 1 x 10^6 P815 or P815 cells transfected with human HLA class I molecules B27 (P815-B27) per ml in RPMI 1640 containing 10% FCS were incubated with 100 μl of serial 4 fold dilutions (1/30 - 1/7680) of monkey plasma (from day of challenge) containing 0.1% sodium azide for 30 min at 4°C. The cells were washed thrice in RPMI with 10% FCS and sodium azide before a further 30 min incubation with 100 μl of 1:100 of rabbit antibody to human immunoglobulin conjugated to FITC (DAKO). The cells were washed as before and resuspended in PBS containing 1% formaldehyde. The percentage and peak channel fluorescence were analyzed on a FACS Consort 30 (Becton Dickinson). The end-point titre was taken as the dilution where $\geq$20% of the cells were positive.

The results are shown in Figure 1. Figures 1a to 1e show that sera from protected monkeys contain antibodies specific for human HLA class I molecule.

a, ^{35}S-methionine labelled C8166 cell lysates were immune precipitated with prebleed (B) and hyperimmune (A) plasma (20μl) from monkeys vaccinated with inactivated purified SIVmac (I179, I180, I181, I182), fixed SIV infected C8166 cells (I217, I219, J68, J69, J71) or C8166 cells (J73, J75). With the exception of J71, J75 and I219, all the other monkeys were protected against SIVmac 251 (grown in C8166 cells) challenge infection.

b, In tracks I181 + W6/32 and anti-β_2m + I182, the radiolabelled lysate was pre-precipitated twice with plasma from I181 or anti-β_2m before subsequent precipitation with mAb W6/32 (specific for a monomorphic determinant on human MHC class I molecule HLA-A, -B, -C) or I182 plasma respectively.

c, Similarly, in track I181 + OKT11, precipitation with mAb OKT11 was subsequent to that with I181 plasma. I181 PB denotes pre-immune plasma.

d, ^{35}S-methionine labelled cell lysates of a Herpes papio transformed monkey B lymphoblastoid cell line were immune precipitated with 25μl prebleed or hyperimmune plasma from protected monkey I181 and 2μl (ascites fluid) of mAb W6/32 or L243 (specific for nonpolymorphic determinants of human class II molecule HLA-DR).

e, FACS analysis of anti-human HLA class I reactivity by prebleed and hyperimmune plasma from a representative monkey, I181, using mouse P815 cells transfected with HLA B27. Results similar to b-e were obtained with sera from other protected monkeys.

2. Detection of Cellular Antigens on SIV and HIV-1 virions by enzyme-linked immunosorbent assay (ELISA)

The 32H cognate isolate of SIVmac 251 and the HIV-1 isolate GB8 were grown in C8166 cells and partially purified by gel exclusion chromatography to minimise loss

of envelope glycoprotein[9]. Both virus preparations were inactivated with formalin and dialysed into phosphate-buffered saline (PBS) before use. For the ELISA assay, 50µl of SIVmac (2µg/ml) or HIV-1 (16µg/ml) diluted in 0.1M carbonate buffer pH 9.6 were added to each well of a 96 well microtitre plate (Nunc, Maxi Sorb) and all subsequent steps carried out as previously described[10].

Monoclonal antibodies (mAbs) and rabbit antibody to human immunoglobulin conjugated to horse radish peroxidase (1:100, DAKO) were diluted in PBS containing 0.05% Tween 20 and 10% heat-inactivated New Born Calf serum (HI NCS). Dilutions of mAb used were 1:100 for ascites fluid, 20-50µg/ml for purified immunoglobulin and neat spent tissue culture supernatant. All washes were with PBS containing 0.05% Tween 20. The substrate used was 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (SIGMA) and the optical density (O.D.) was measured at 405nm.

The results are shown in Table 1. Values (mean ± 1 SEM, N=6) that were five times higher than the negative control reading were considered as positive (in bold type). The mAbs used were obtained from the AIDS directed programme, MRC (ADP373, ADP317, ADP318, ADP336, ADP351, ADP356, ADP359); the American Type Tissue Culture Collection (W6/32, L203, L227, L243, OKT3); Bristol-Myers Squibb, Seattle (9.3) and OKT11 and nm31 were kind gifts from Dr. D. Cantrell, ICRF, Lincoln's Inn Field, London and Prof. A. McMichael, Institute for Molecular Medicine, Oxford respectively. All the mAb used have been checked

for activity using flow cytometry (data not shown). Table 1.

Antibody	Antigen recognised	O.D. Reading SIV	HIV
None	-	0.06 ± 0.007	0.014 ± 0.003
ADP373 IgG_{2a}	SIV env gp160	0.344 ± 0.025	ND
ADP317 IgG_3	HIV-1 env gp160	ND	0.116 ± 0.005
W6/32 IgG_{2a}	HLA-A,-B,-C	0.333 ± 0.038	0.113 ± 0.012
OKT3 IgG_{2a}	CD3	0.314 ± 0.06	0.323 ± 0.008
L203 IgG_1	HLA Class II	0.017 ± 0.001	0.012 ± 0.004
L227 IgG_1	HLA Class II	0.017 ± 0.001	0.008 ± 0.001
L243 IgG_{2a}	HLA Class II	0.05 ± 0.009	0.058 ± 0.01
nm31 IgG	CD2	0.036 ± 0.004	0.032 ± 0.008
OKT11 IgG_{2a}	CD28	0.043 ± 0.009	0.027 ± 0.003
9.3 IgG_{2a}	CD4	0.03 ± 0.002	0.014 ± 0.002
ADP318 IgG_1	CD4	0.014 ± 0.002	0.011 ± 0.006
ADP336 IgG_{2a}	CD4	0.058 ± 0.006	0.062 ± 0.002
ADP351 IgG_{2a}	CD4	0.025 ± 0.003	0.020 ± 0.008
ADP356 IgG_{2b}	CD4	0.036 ± 0.002	0.033 ± 0.001
ADP359 IgG		0.016 ± 0.001	0.007 ± 0.001

3. Inhibition Xenoantigen-driven Monkey T Cell Proliferation by Sera from Protected Monkeys

Monkey PBMC were isolated from defibrinated blood (obtained from monkeys vaccinated with SIV env) by centrifugation on percoll gradients. PBMC at 1 x 10^6 per ml RPMI 1640 containing 10% autologous serum and 2-mercaptoethanol (5 x 10^{-5}M) were dispensed at 100μl/well

into a 96 well flat-bottomed plate (Costar).

Then 50µl of the indicated dilutions of sera (all heat inactivated at 56°C for 45 min) from protected (P) or not protected (NP) monkey or medium were added to the wells. This was followed by the addition of 50µl of SIV gp140 (Repligen, 5µg/ml). In the mixed lymphocyte cultures, 50µl containing 1 x 10^5 irradiated C8166 cells (7500 R) or PBMC from a naive monkey J7 (2500 R) were added as stimulating antigen.

The cultures were incubated at 37°C in a humidified CO_2 incubator for 5 days and were pulsed with 1µCi/well ^{3}H-thymidine (Amersham, specific activity 25Ci/mmol) during the final 6 hr of incubation. Cultures in triplicates were harvested on an automatic cell harvester (LKB, Sweden) and radioactivity counted in a β counter (LKB, Betaplate, Sweden). In the adsorption experiments, 700µl of 1:40 diluted monkey sera were incubated with 10^7 P815 or P815-A1 cells followed by a second incubation with 10^7 P815 or P815-B27 cells respectively.

The preabsorbed sera were filtered (0.22µm) and appropriately diluted before adding to the cultures. The results are shown in Table 2. Each value represents mean ± 1 SEM, N=3. Those values showing ≥75% inhibition are indicated in bold type. Similar results were also obtained with other sera and monkey PBMC.

Table 2

		[^{3}H]TdR incorporation (cpm x 10^3)		
Antibody		C8166	J7 PBMC	rgp140
Expt. 1				
None		200.0 ± 12.4	137.8 ± 6.7	16.1 ± 2.0
I181 (P)	1:320	7.5 ± 1.0	129.3 ± 4.3	14.5 ± 2.9
	1:640	28.2 ± 5.4	141.6 ± 20.1	ND
	1:2560	149.4 ± 4.3	132.9 ± 2.7	ND
J140 (P)	1:320	8.3 ± 1.0	116.7 ± 10.8	13.3 ± 3.1
	1:640	48.4 ± 1.0	113.6 ± 9.6	ND
	1:2560	151.9 ± 7.6	118.2 ± 5.4	ND
J139 (NP)	1:320	152.1 ± 2.1	106.0 ± 1.1	12.7 ± 0.3
	1:640	152.6 ± 20.6	134.8 ± 17.4	ND
Expt. 2				
None		93.1 ± 3.0		
I181	1:320	11.3 ± 1.3		
	1:640	40.6 ± 7.8		
	1:2560	57.5 ± 1.9		
I181+p815-A1	1:320	65.5 ± 10.1		
+P815-B27	1:640	77.3 ± 11.5		
	1:2560	84.3 ± 5.5		
	1:320	20.5 ± 1.2		
	1:640	56.6 ± 6.0		
	1:2560	67.9 ± 6.5		

4. Inhibition of PHA, OKT3 or Antigen-driven Human T Cell Proliferation by Sera from Protected Monkeys

Human PBMC were isolated from defibrinated blood (obtained from an individual vaccinated 4 times with botulinum toxoid) by centrifugation on Histopaque (SIGMA) gradients. The assay for T cell proliferation is essentially the same as in Table 2 except that Phytohaemagglutinin (PHA, Wellcome Diagnostics, 11μg/ml) or botulinum toxoid (obtained from Dr. D. Sesardic, 2.5μg/ml) or purified OKT3 (5ng/ml) were used to stimulate the T cells. Ascites fluid of mAbs W6/32 and ADP 314 (anti-HIV-1 p55/p24) were also used as control antibodies. The cultures were incubated for 3 days in the case of cultures with PHA or OKT3 and 5 days with antigen. The results are shown in Table 3. Each value represents mean ± 1 SEM, N=3. Those values showing ≥75% inhibition are indicated in bold type. Similar results were also obtained with other monkey sera. Control monkey J142 was vaccinated with RIBI adjuvant only.

Table 3

			[^{3}H]TdR incorporation (cpm x 10^3)	
Antibody		PHA	OKT3	BIxD
Expt. 1				
None		42.0 ± 4.3	75.0 ± 2.2	
W6/32	1:400	1.5 ± 0.1	0.12 ± 0.01	
	1:2000	12.7 ± 2.0	0.80 ± 0.12	
	1:10000	40.6 ± 1.3	42.0 ± 2.8	
ADP314	1:400	33.1 ± 3.0	70.1 ± 2.1	
I180(P)	1:800	4.4 ± 0.8	0.47 ± 0.04	
	1:4000	41.6 ± 3.0	9.17 ± 3.7	
	1:20000	32.3 ± 1.9	45.81 ± 2.8	
I181(P)	1:800	0.3 ± 0.1	0.24 ± 0.02	
	1:4000	22.7 ± 4.0	1.56 ± 0.37	
	1:20000	45.7 ± 10.8	31.77 ± 2.1	
Preimmune	1:800	43.5 ± 10.4	80.57 ± 2.2	
J142 (NP)	1:800	55.0 ± 10.4	83.07 ± 4.6	
Expt. 2				
None			23.4 ± 2.0	19.3 ± 3.4
I181 (P)	1:800		ND	0.3 ± 0.1
	1:4000		ND	18.2 ± 1.2
J141 (P)	1:800		0.63 ± 0.1	4.6 ± 1.9
	1:3200		4.23 ± 0.6	12.7 ± 1.1
	1:12800		ND	19.1 ± 3.3
J139 (NP)	1:800		9.18 ± 1.1	16.2 ± 0.8
	1:3200		21.5 ± 1.5	17.8 ± 3.5
J142 (NP)	1:800		ND	18.7 ± 3.6

5. Discussion

Radioimmune precipitation of ^{35}S-methionine labelled C8166 cell lysate with the monkey sera demonstrate that all the sera from protected but not from unprotected monkeys recognise two major protein bands, 12 and 44kDa (Fig. 1a). These bands were precipitated by sera from all the protected animals in the three vaccine groups but not by their preimmune sera. Blocking experiments were carried out with ^{35}S-methionine labelled C8166 cell lysate by pre-precipitation with serum from protected monkeys followed by precipitation with mAb W6/32 (specific for a monomorphic determinant on human MHC class I molecule HLA-A, - B, -C). Alternatively, the lysate was pre-precipitated with rabbit anti-β_2m (the β chain of HLA class I molecule) followed by precipitation with serum from protected monkeys.

Results shown in Fig. 1b demonstrate that the 12 and the 44kDa bands are β_2m and the heavy (h) chain of HLA class I molecule respectively. Flow cytometry analysis also shows that sera from protected monkeys recognised a murine cell line (P815) transfected with human HLA class I B27 (Fig 1e); mean antibody titre of protected monkeys was $\log_{10}$ 3.1 $\pm$ 0.13 (N=32), and of the unprotected group was 1.86 $\pm$ 0.09 (N=11), P< 0.0005. The intensity and mass of the bands around the 44kDa region precipitated by sera from some protected monkeys (Fig. 1a) suggest that other T cell surface proteins with similar molecular weights such as CD28 (44kDa), CD2 (T11, 50kDa) and actin (44kDa) may also be recognised.

However, the sera from protected monkeys vaccinated with purified SIVmac 251 virus do not contain anti-CD2 (Fig. 1c) or anti-CD28 antibodies (data not shown). The sera from protected monkeys did not precipitate the α-chain or the β-chain of the class I molecule of ^{35}S-methionine labelled, Herpes papio-transformed monkey B lymphoblastoid cell lines (Fig. 1d), suggesting that the anti-human class I antibodies in the sera are directed at polymorphic

regions of the human HLA class I molecule.

The high levels of anti-human HLA class I antibody induced in protected monkeys vaccinated with purified SIVmac 251 virus suggest that the purified virus preparation may contain HLA class I antigen. Our ELISA results (Table 1) confirm that the partially purified SIVmac 251 virus preparation used for immunisation, and a HIV vaccine preparation (GB8), contain a HLA class I molecule and, additionally, CD3, a T cell antigen forming part of the T cell receptor (TcR) complex, but little or no detectable class II, CD4, CD2 or CD28 antigens. However, sera from protected monkeys were not able to precipitate any bands depicting the CD3 γ-, δ-, ϵ-, ζ-, and η- chains (26, 20 and 16kDa), (Fig. 1a) indicating that CD3 was poorly immunogenic in these monkeys that the level of anti-CD3, if present is below the limit of our assay system.

Several reports have shown that mAb to HLA class I heavy chain or to β_2m can inhibit T cell proliferation and activation in vitro[11-19]. In addition, these antibodies are able to inhibit HIV replication in vitro[20-22]. We investigated the effect of sera from protected and unprotected monkeys on the proliferative response of peripheral blood T cells from monkeys to irradiated C8166 cells or monkey PMBC or a recombinant SIV antigen (rgp140) in vitro (Table 2). Sera from unprotected monkeys had no effect on any of the proliferative responses. Although sera from protected monkeys had no effect on the proliferative responses to irradiated monkey cells or rgp140, they were strongly inhibitory to the proliferative response of monkey T cells to C8166 cells. The inhibitory effect of the sera was significantly removed by adsorption with P815 cells transfected with human HLA class I genes (A1 and B27) but not with P815 cells alone (Table 2).

We also investigated the effect of the sera from protected monkeys on the proliferative responses of human peripheral blood T cells to phytohaemagglutinin (PHA),

anti-CD3 antibody (OK3) and a specific antigen, botulinum toxoid (BTxd) (Table 3). Sera from unprotected monkeys had no effect on the antigen-specific proliferation, and only a weak inhibitory effect (J139), vaccinated with SIV) on OKT3-driven response at high concentrations. In contrast, sera from protected monkeys and the mAb W6/32 strongly inhibited all these response. The inhibitory effects of these sera were again removed by adsorption with P815 cells transfected with human HLA class I genes (A1 and B27) (data not shown).

Our results demonstrate a direct correlation between antibody response to HLA class I antigen and protection of monkeys from SIVmac 251 (grown in C8166 cells) infection. The precise mechanism of the possible protection by this antibody is at present unclear. It may be that the antibody reacts with the class I antigens in the virus envelope and thereby blocks the interaction of gp120 with the CD4 determinants on the target cells by steric hindrance. However, this seems unlikely since sera from protected monkeys did not block the binding of live SIV to CD4-bearing human T cells[23].

Furthermore, sera of unprotected monkeys contain high levels of specific anti-SIV antibody [3,4,6,23,24] indicating that blocking virus-CD4 interaction alone may not be a major protective mechanism in the present system. The presence of high levels of SIV-specific antibody in unprotected monkeys also argues against the notion that cross reaction between class I antigens and lentivirus may play an important role in protection against SIV infection[25].

However, it does not rule out the possibility that specific neutralising antibody could protect monkeys against a homologous challenge infection with cloned SIV[26]. It is also unlikely that anti-CD4 antibody plays a significant role in the present system since no anti-CD4 antibody was detected in the sera of protected monkeys by

flow cytometry (data not shown), nor is CD4 antigen present in the SIV virus preparation (Table 1). In contrast to a previous report[27], we failed to detect class II antigens in our SIV virus preparation. This may be due to the different procedures used in purifying the viruses.

Our data suggest that the anti-class I antibody may contribute towards the protection against SIV infection by down-regulating T cell activation, which is known to be required for HIV replication [28-34]. This is consistent with previous reports that anti-class I antibody can prevent HIV replication in human PMBC[20-22]. The precise mechanism by which anti-class I antibodies down regulate T cell activation is unknown. They may act by inhibiting monocyte antigen presentation. Therefore when protected monkeys were challenged with SIV preparations containing human class I antigens, the anti-human class I antibody could bind and effectively block these antigens from reacting with the monkey antigen presenting cells, thereby preventing the dominating xenogenic activation (>10x higher than anti-SIV response, Table 2) of T cells and hence inhibiting virus replication.

Since the anti-human class I antibody does not cross-react with the monkey class I antigens (Fig. 1d), it would not block the monkey class I antigens (present in SIV grown in monkey cells) from binding to the monkey antigen presenting cells (Table 2) when the vaccinated monkeys were challenged with that virus. Thus there is no down-regulation of allogenic activation and so virus replication is not inhibited. This may explain the finding that vaccinated monkeys were not protected against challenge infection with SIV grown in monkey cells [4-6].

Another way by which anti-class I antibody can down-regulate T cell activation is by acting directly on the class I molecules on T cells [19]. The anti-human class I antibody from protected monkeys would not be expected to down-regulate the activation of monkey T cells induced by

specific antigen by this route, because of the lack of cross-reactivity between the antibody and monkey class I antigens on monkey T cells. Since SIV antigen induced activation is at least 10X weaker than xenogenic or allogenic activation (Table 2), the T cell activation so induced may not be sufficient to support adequate virus replication to infect the monkeys. However, the antibody would be expected to strongly inhibit the activation of human T cells to specific antigens or mitogens. This is indeed the case in experiments presented in Table 3. The finding suggests that anti-human HLA class I antibodies may be useful in immunotherapy against HIV infection.

Although our data suggest that the anti-class I antibody contributes towards the resistance to SIV infection in $CD4^+$ T lymphocytes, it may not have an effect on the infection of monocytes. In any case, the virus is not expected to replicate extensively in these cells, and virus grown in T cells or PHA activated PBMC would only infect the homologous cell type [35].

EXAMPLE 2

Four macaques were vaccinated with a mixture of murine P815 cells transfected with HLA class I (B27) and purified HLA A1 and B8 (the haplotype of C8166 cells) from a human B cell line as immunogen and using GMDP formulated with squalane and pluronic-in-water emulsion as adjuvant. Control monkeys were immunised with P815 cells and adjuvant. After the second vaccination, all 4 vaccinees showed good antibody response (>1/120) by flow cytometry on P815-B27 cells and on the human B cell line from which the HLA immunogen was prepared. Control animals had antibody titres of <1/30. After the third vaccination, the antibody titres in the vaccinees increased further to $\geq$1/1920 when assayed by flow cytometry on both P815-B27 and C8166 cells. The antibody titre in control animals remained at <1/30. Using radioimmunoassay with purified HLA I adsorbed to

plastic, the vaccinees had antibody titres of 1/120 - 1/480 whereas that of control animals and prebleeds was <1/30. Sera from the vaccinees but not the control animals were also shown to bind to the SIV virus envelope as demonstrated by immuno-gold labelling under electron microscopy.

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CLAIMS

1. A major histocompatibility complex class I antigen for use in a method of treatment of the human or animal body by therapy.

2. An antigen according to claim 1 for use as a vaccine against an immunodeficiency virus.

3. An antigen according to claim 2, wherein the virus is a human immunodeficiency virus(HIV).

4. An antigen according to claim 3, wherein the virus is HIV-1.

5. An antigen according to claim 3, wherein the virus is HIV-2.

6. An antigen according to any one of the preceding claims, which is a human class I antigen.

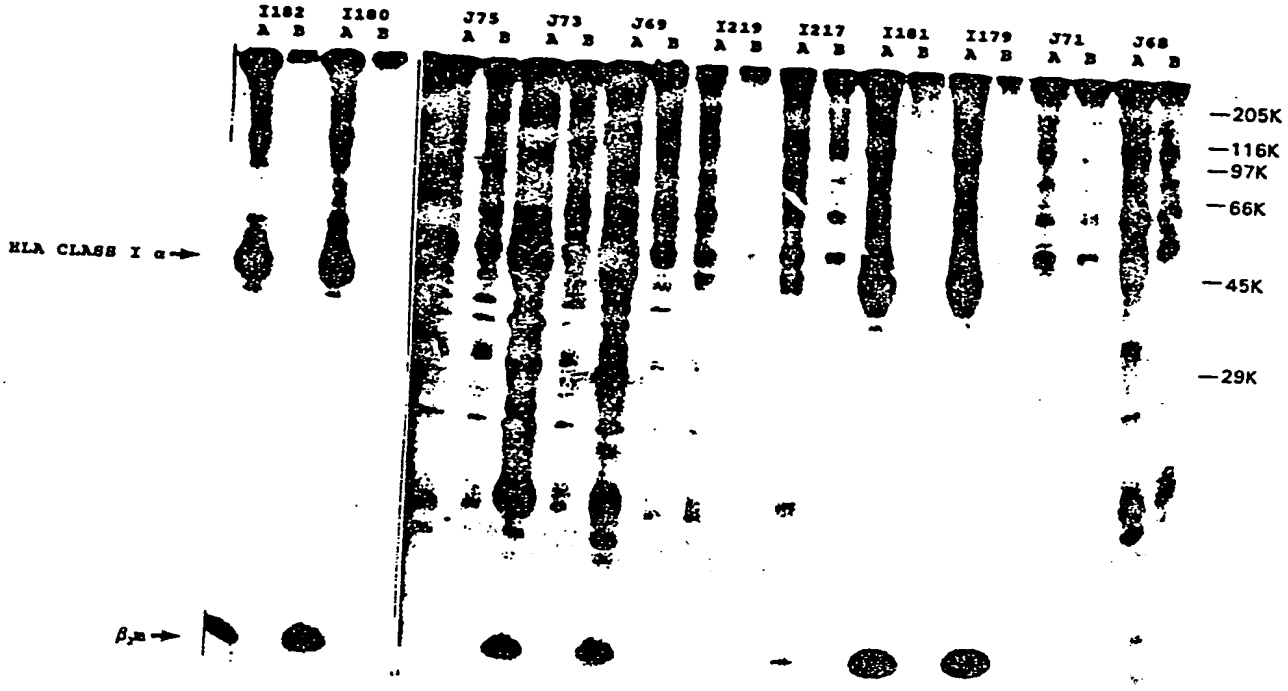
7. An antigen according to claim 6, which is a HLA-A, HLA-B or HLA-C antigen or β_2 microglobulin.

8. A pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and, as active ingredient, a major histocompatibility complex class I antigen.

9. Use of a major histocompatibility complex class I antigen in the manufacture of a medicament for use as a vaccine against an immunodeficiency virus.

10. A method of vaccinating a host against an immunodeficiency virus, which method comprises administering to the host an effective amount of a major histocompability complex class I antigen.

11. An agent useful as a vaccine against an immunodeficiency virus, which agent comprises a major histocompatibility class I antigen.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I180 A B
J75 A B
J73 A B
J69 A B
I219 A B
I217 A B
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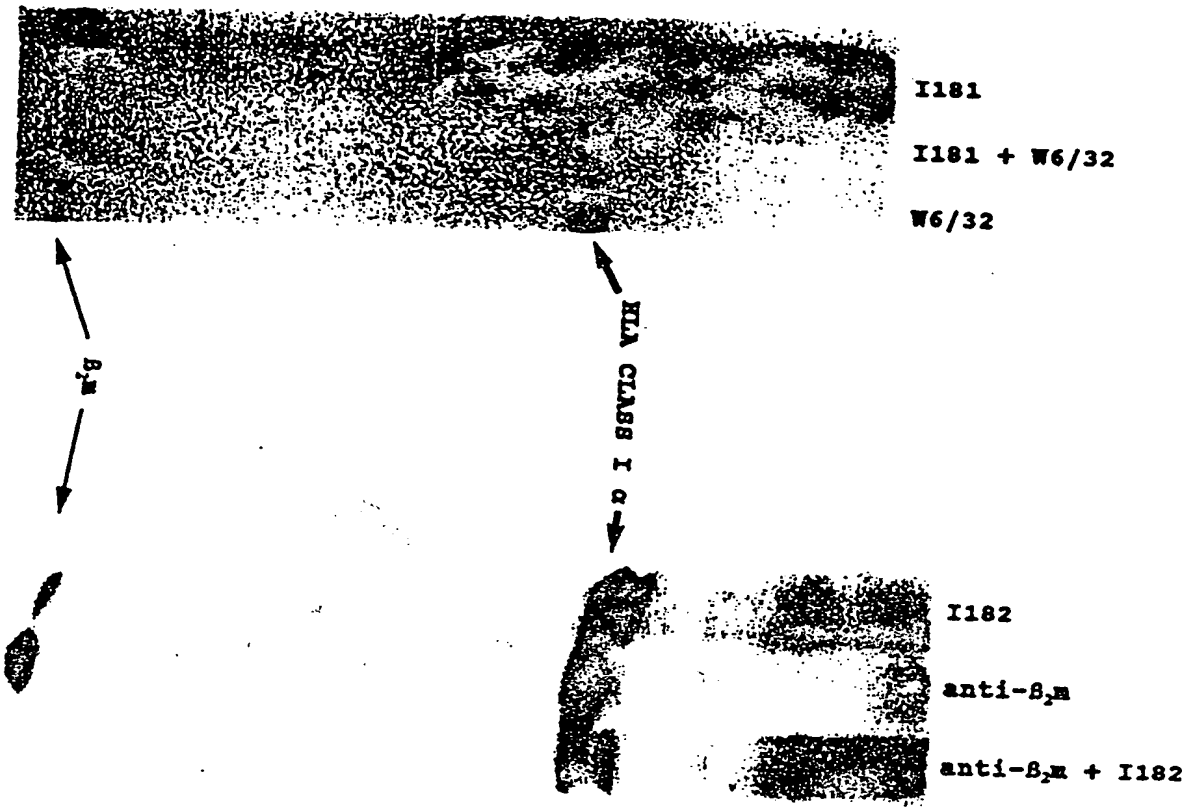
I179 A B
J71 A B
J68 A B
HLA CLASS I α
β_2m
205K
116K
97K
66K
45K
29K

Fig 1A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I181 + W6/32
W6/32
β_2m
HLA CLASS I α
I182
anti-β_2m
anti-β_2m + I182

Fig 1B

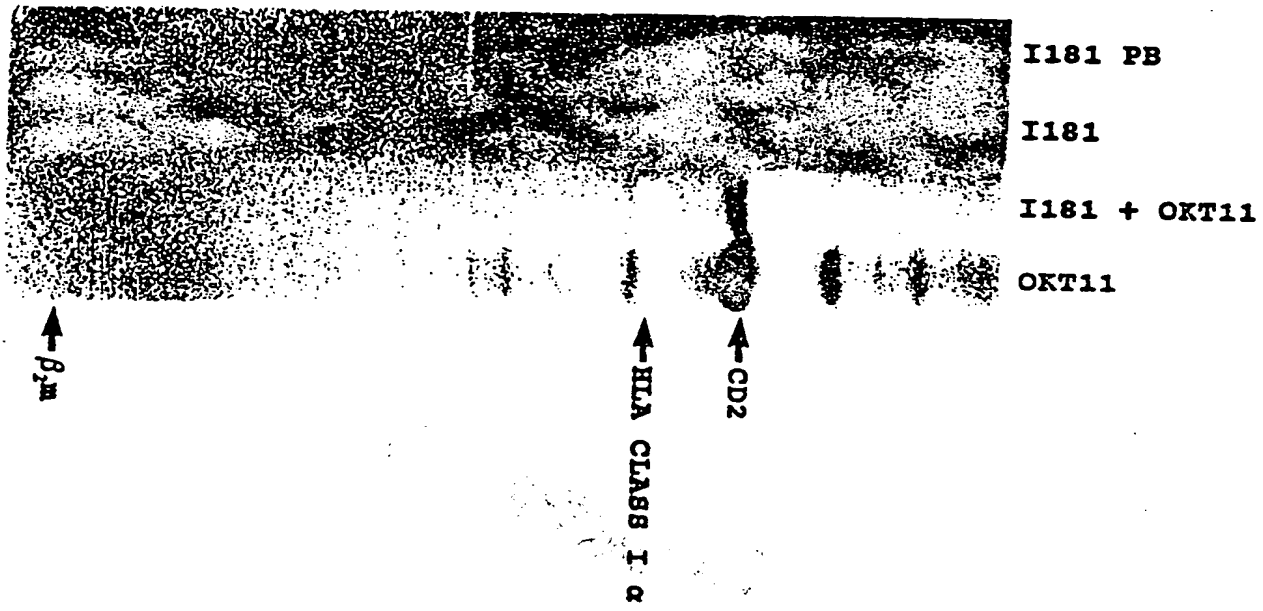
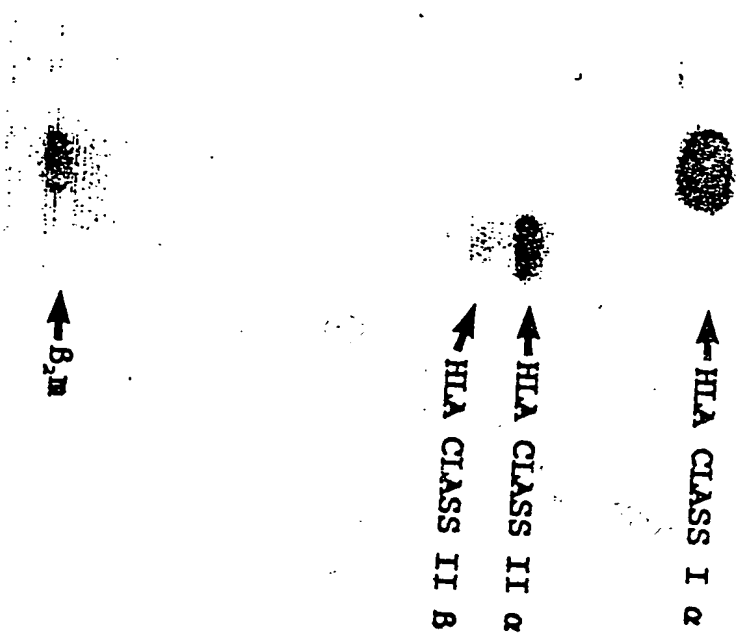


Fig 1C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I181
W6/32
L243
β_2m
HLA CLASS II β
HLA CLASS II α
HLA CLASS I α

Fig 1D

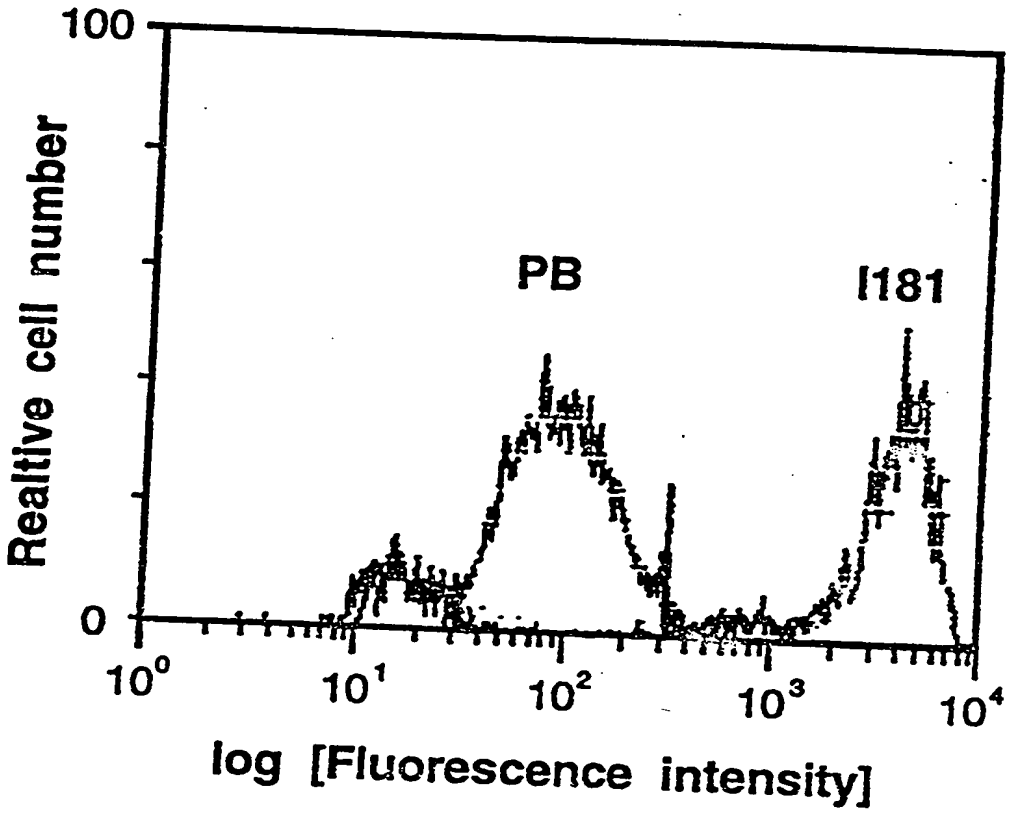
100
0
Realtive cell number
PB
I181
10^0
10^1
10^2
10^3
10^4
log [Fluorescence intensity]

Fig 1E

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

GB 9301459
SA 76522

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 12/10/93

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9116924	14-11-91	AU-A- 7987391	27-11-91
		EP-A- 0531401	17-03-93

INTERNATIONAL SEARCH REPORT

Int ...ional application No.
PCT/GB93/01459

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark : Although claim 10 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition..

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
P,X	THE JOURNAL OF EXPERIMENTAL MEDICINE vol. 176, no. 4, 1 October 1992, NEW YORK, USA pages 1203 - 1207 W-L. CHAN ET AL. 'Protection in simian immunodeficiency virus-vaccinated monkeys correlates with anti-HLA class I antibody response.' see page 1206, left column, line 28 - right column, line 43	1-11

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 93/01459

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61K39/00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	WO,A,9 116 924 (THE DANA-FARBER CANCER INSTITUTE) 14 November 1991 see page 21, line 26 - line 27; claims	1-11
A	IMMUNOLOGY LETTERS vol. 24, no. 2, May 1990, AMSTERDAM, THE NETHERLANDS pages 127 - 132 D. LEWIS ET AL. 'HLA-DR peptide inhibits HIV-induced syncytia.' see abstract	1-11

-/--

* Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
12 OCTOBER 1993	20 -10- 1993
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	NOOIJ F.J.M.